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# ON THE RELATION OF URIC ACID EXCRETION TO DIET.

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(Ten Figures in Text.)

(*From the Physiological Laboratories, Guy's Hospital.*)

Experiments carried out more than ten years ago by F. Mareš[1] showed that during the period of increased nitrogen excretion which follows an isolated meal, the increase of uric acid has a briefer duration than the increase of urea, and occurs, characteristically, in quite the earliest hours of the hyper-excretory period. Mareš found that while the uric acid rises immediately after the meal, attains its maximum at about the fifth hour, and sinks again rapidly, the urea increases more slowly, is not at its height until about the ninth hour, and makes a slow return to the value it had before the meal.

Mareš believed that the explanation of this want of parallelism was to be found in the fact that, while the urea arises directly from the ingesta, the uric acid takes origin from the tissues, and is increased by a meal only because of increased or modified cellular activity during digestion. By Horbaczewski this view was made more special and determinate in the well-known and widely accepted theory which attributes the increase of uric acid after food to the occurrence of a digestive leucocytosis and the consequent increased liberation of nucleins within the body.

The belief that uric acid has this special origin (off the lines, so to speak, of general metabolism) predisposes to the acceptance of statements like those of Mareš, which ascribe to it a special period and rate of excretion, independent of the main nitrogenous excretory tide. This is probably why the otherwise striking results obtained by this observer have not been submitted to the test of repetition.

But the theory that a meal increases the output of uric acid exclusively through its influence on the leucocytes of the blood stream has not remained without challenge. Mareš[2] himself was an early opponent of the view. He held that all Horbaczewski could be said

[1] *Arch. slave de biol.* III. p. 207. 1887.

[2] *Monatsh. f. Chemie*, XIII. p. 101. 1892.

to have proved experimentally was that under special circumstances an increase of uric acid in the urine runs parallel with an increase of leucocytes in the blood. For the theory to hold good, destruction of these cells and the liberation of nucleins or precursors of uric acid must be proved to go on simultaneously, and that this actually occurs Mareš held to be a hypothesis only[1].

But much more direct evidence against the existence of any universal causal relation between digestive leucocytosis and uric acid increase is obtained from experiments in which a special dietary is used. We shall quote experiments later which show that under some circumstances a well-marked digestive leucocytosis may occur after a non-nitrogenous meal, which is quite unassociated with any rise of uric acid. Many observers have found that after the ingestion of egg-white, there is little or no rise of the acid[2], while others have shown that the digestion of this proteid is associated with marked increase in the circulating leucocytes[3].

This subject is dealt with further in a special section of this paper. It is sufficient to remark here that such facts as those already detailed have led many to look upon the post-prandial increase of uric acid as originating directly from the nucleins taken with the meal; and the correctness of this view has been held proved by the familiar experiments which have demonstrated the occurrence of a strikingly large increase of the uric acid during the use of thymus-gland as a diet. The uric acid is looked upon in this later view as arising immediately from the breakdown of the ingested nucleins independently of any intermediary leucocytosis.

But if this be so the observations of Mareš become of greater interest; for it is remarkable that an excretive taking origin from the least digestible of the proteid constituents of the diet,—upon which gastric digestion itself has but a minimal influence,—should appear during the earlier, rather than during the later, hours of the post-prandial nitrogen tide. It seemed to us therefore of some importance to repeat the experiments of Mareš upon a larger number of individuals. His results if established would show that the digestion of nucleins in the body differs greatly from the process in vitro, or would suggest that the breakdown of these special proteids is not the whole history of uric acid production.

[1] Cf. Milroy and Malcolm. This *Journal*, XXIII. p. 218. 1898.
[2] Hess and Schmoll. *Arch. f. exp. Path. u. Phar.* XXXVII. p. 243. 1896.
[3] Pohl. *Ibid.* XXV. pp. 31—50.

*Methods used in this research.* In estimating uric acid Mareš used the Salkowski-Ludwig process; his figures are therefore beyond reproach. But his observations were made upon a few individuals only, and upon the urine of consecutive three-hourly periods—intervals too long to permit of the relative rates of excretion of urea and uric acid being accurately followed. In his experiments, moreover, abnormally large meals of proteid were taken.

In such of our own experiments as were directed to a repetition of the work of Mareš, we kept the following ends in view:—(1) To employ as many individuals as possible, in order that the effects of personal idiosyncrasy might be eliminated. Altogether the sequence of excretion was studied in seven persons (adult males). (2) To test the effect of a meal fully isolated, and so to avoid any interference from the remains of the nitrogenous tide due to previous food ingestion. Mareš believed that the excretion of uric acid remained at a nearly constant level from the 13th to the 24th hour of fasting. In our experiments no food was taken after dinner at about 7 p.m., until the test meal was taken at about 1 p.m. on the following day. (3) To test the effect of meals of ordinary mixed diet and of normal dimensions. In general each individual took such a meal as was customary to his habits and appetite. Except for an effect of the preliminary abstinence the conditions of digestion were therefore as normal as possible. The food usually consisted of beef-steak, bread and potatoes. The actual amount of nitrogen ingested was without importance in this part of our enquiry, and was not determined. In later experiments with other bearings, the nitrogen was, when necessary, determined by Kjeldahl's method. (4) To make the intervals at which consecutive determinations were made as short as possible. The urine was collected at hourly intervals, generally during the three hours before the meal and up to about the tenth hour after it; determinations being made on each hourly quantity. No food was taken after the test meal until the experiment was finished.

The uric acid was determined throughout by the process described by one of us, in which it precipitated as acid ammonium urate by saturating the urine with ammonium chloride; the acid itself being subsequently liberated and titrated with standard permanganate solution. Many observers have now compared this method with the standard Salkowski-Ludwig process and always with favourable results[1].

[1] Cf. Huppert. *Analyse des Harns*, p. 820, Wiesbaden, 1898.

We therefore felt justified in relying upon the method in view of its great convenience where a considerable number of estimations have to be made in one series. Once saturated with the ammonium salt, the urine, if protected from evaporation, may be left almost indefinitely; no decomposition occurs, and nothing beyond the original ammonium urate precipitate comes down, so that the completion of the process may be carried out at leisure. It is quite possible with this process to obtain accurate results from the small quantities of urine necessitated by hourly observations.

The urea excretion was followed for the most part by means of the Knop-Hüfner process. In one experiment the total nitrogen was determined from hour to hour by Kjeldahl's process; and in another the series of urea estimations was made by the Mörner-Sjöqvist method. But the curves from results so obtained did not differ essentially from those given by the figures of the hypobromite process; and the extra labour involved yields no advantage in such an enquiry as ours, where an efficient measure of the hourly variations in the main nitrogenous excretion to contrast with the uric acid output is all that is requisite. Exact uniformity of the conditions under which the hypobromite-nitrogen was measured was carefully maintained throughout each series.

## I. The relative rates of excretion of urea and uric acid after a meal.

The results of our observations upon the effect of isolated meals of mixed diet amply bear out the statements of Mareš as regards the proportionately early appearance of uric acid in the post-prandial excretory tide. Its increase frequently begins before that of the urea, and is at its greatest during the earlier hours of digestion. On the other hand it usually falls to its abstinence value some hours before the urea tide is exhausted, and in no single experiment have we found its increase to outlast the latter.

In our experiments the uric acid maximum was generally found at the 3rd or 4th hour after the meal, that of the urea showing little regularity of incidence though generally occurring later[1]. At times the actual maximum of urea may fall early and may then coincide with that of the uric acid; but even in such cases the proportionate increase

[1] Our results seem to show that the regular incidence of maxima in the course of urea excretion after a meal, described by Tschlenoff (*Correspondenz-Blatt f. Schweizer Aerzte*, No. 3, 1896) and by Veraguth (this *Journal*, xxi. p. 112, 1897), is not to be observed in all individuals.

of the latter is at this early period (2nd to 5th hour) much greater, so that the ratio it bears to the urea is markedly raised; whereas later (5th to 10th hour), while the urea is still maintained above the level of the abstinence period, the uric acid falls to its abstinence value or even below this. A curve of the uric acid values expressed as fractions of the urea values (ratio curve) shows therefore, in general, a sharp rise soon after the meal, but during the later hours of digestion it drops below the level found before the meal.

Exp. I. (F. H. April 24, 1896.) Abstained from 7 p.m. April 23. At 1 p.m. April 24, took 250 grms. beef (weighed raw) with 100 grms. bread.

| Hour | Quantity of urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio uric acid : urea |
|---|---|---|---|---|
| 10—11 a.m. | 60 | 0·91 | 23 | 1 : 40 |
| 11—12 | 48 | 0·78 | 25 | 1 : 31 |
| 12— 1 p.m. | 55 | 0·81 | 22 | 1 : 37 |
| 1— 2 | 55 | 0·70 | 30 | 1 : 23 |
| 2— 3 | 48 | 0·88 | 42 | 1 : 21 |
| 3— 4 | 85 | 0·91 | 52 | 1 : 18 |
| 4— 5 | 54 | 1·25 | 40 | 1 : 31 |
| 5— 6 | 58 | 1·78 | 44 | 1 : 40 |
| 6— 7 | 60 | 1·56 | 38 | 1 : 41 |
| 7— 8 | 49 | 1·05 | 29 | 1 : 36 |
| 8— 9 | 51 | 1·10 | 24 | 1 : 46 |
| 9—10 | 45 | 1·42 | 24 | 1 : 59 |
| 10—11 | 36 | 0·98 | 20 | 1 : 49 |
| 11—12 | 38 | 0·98 | 16 | 1 : 61 |

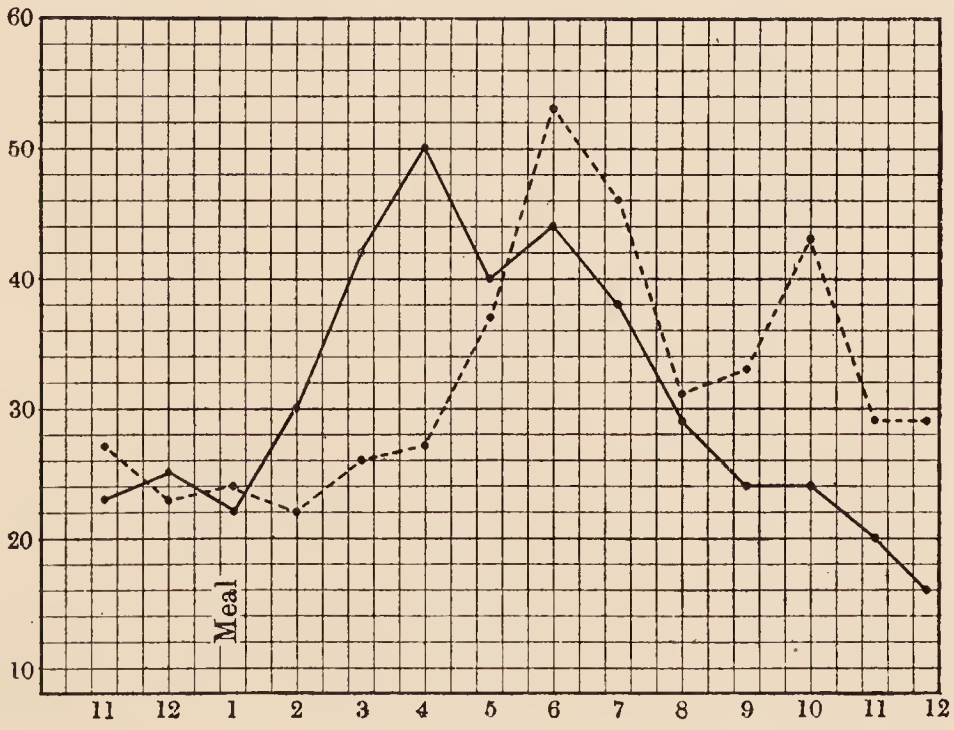


Fig. 1. Curves of Urea and Uric Acid[1]. Exp. I.

[1] In this curve and in those which follow the continuous line represents Uric Acid and the dotted line Urea. The uric acid curve represents its values in milligrammes; that of


Fig. 2. Ratio Curve. Exp. I. (Uric Acid in percentages of the Urea.)

In Experiment II. the uric acid shows a sharp rise with a maximum at the 4th hour after the meal. The urea fluctuations are irregular but an increase is maintained long after the uric acid has fallen to the abstinence value.

Exp. II. (W. H. Nov. 10, 1897.) Abstained from 7 p.m. Nov. 9. At 1.30 p.m. Nov. 10, took 200 grms. of beef-steak with small quantities of potatoes and bread.

| Hour | Quantity of urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio uric acid : urea |
|---|---|---|---|---|
| 10—11 a.m. | 175 | 1·07 | 26 | 1 : 41 |
| 11—12 | 118 | 1·13 | 27 | 1 : 42 |
| 12— 1 p.m. | 164 | 1·07 | 24 | 1 : 45 |
| 1— 2 | 60 | 0·64 | 21 | 1 : 30 |
| 2 — 3 | 43 | 1·12 | 22 | 1 : 50 |
| 3— 4 | 41 | 1·16 | 38 | 1 : 31 |
| 4— 5 | 53 | 0·84 | 40 | 1 : 21 |
| 5— 6 | 59 | 1·16 | 56 | 1 : 21 |
| 6— 7 | 56 | 1·20 | 39 | 1 : 30 |
| 7— 8 | 95 | 1·37 | 30 | 1 : 46 |
| 8— 9 | 183 | 1·49 | 33 | 1 : 45 |
| 9—10 | 155 | 1·33 | 24 | 1 : 55 |
| 10—11 | 180 | 1·33 | 23 | 1 : 56 |
| 11—12 | 96 | 1·37 | 22 | 1 : 62 |

urea is plotted from empirical figures obtained by dividing the values in milligrammes by 33·3. Where the curves meet therefore the two excretives are present in what is frequently spoken of as the 'normal' ratio.

The 'ratio' curves show the proportion which uric acid bears to urea from hour to hour; they are plotted from the values of the former expressed as percentages of the values of the latter.

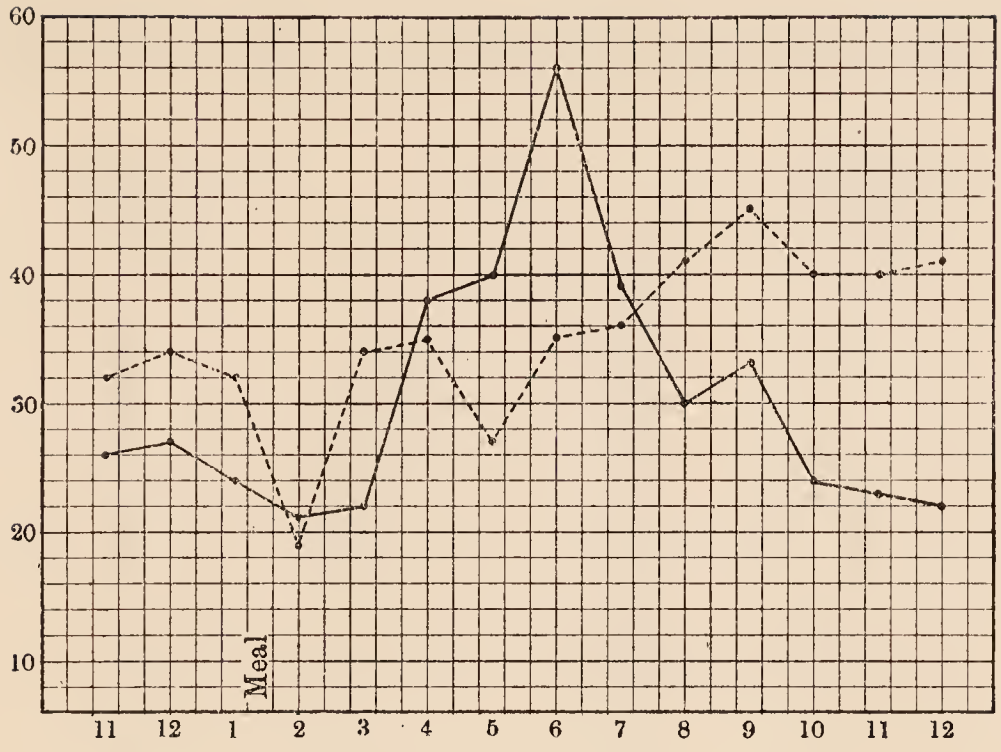


Fig. 3. Urea and Uric Acid Curves. Exp. II.

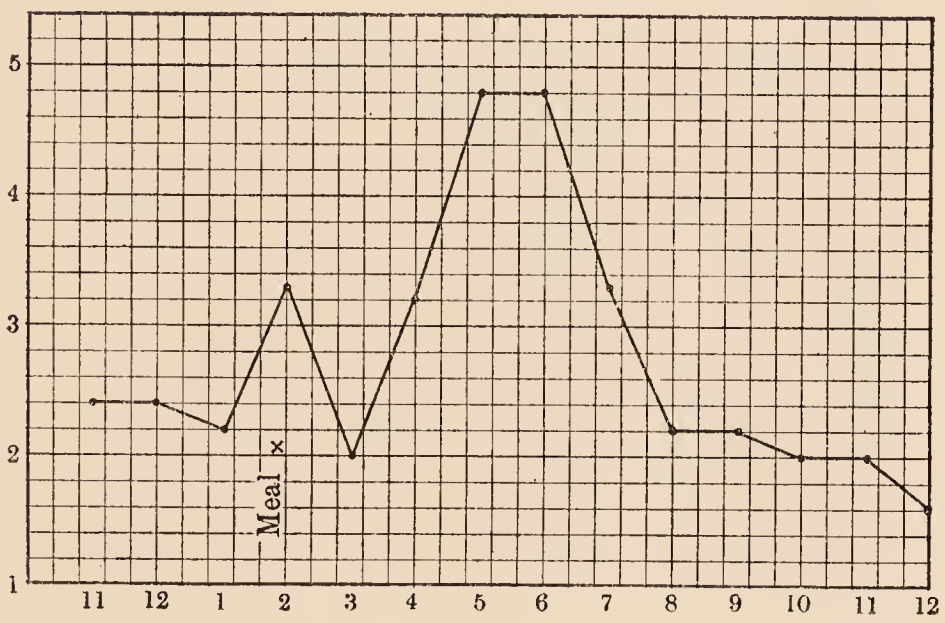


Fig. 4. Ratio Curve. Exp. II.

In the following experiment the maxima of both excretives occur together at the 4th hour, but the urea shows later rises at the 7th and 9th hours while the uric acid is then below its abstinence value. From the 1st to the 4th hour the uric acid bears a high ratio to the urea, from the 5th to the 10th hour a low one.

Exp. III. (E. L. May 6, 1896.) Abstained from 7.30 p.m. May 5. At 12.40 p.m. May 6, took meal of beef and bread. At 6 p.m. a little tea was drunk.

| Hour | Quantity of urine in c.c. | Acidity | Urea in grms. | Uric acid in milligrms. | Ratio uric acid : urea |
|---|---|---|---|---|---|
| 10—11 a.m. | 55 | 15·3 | 0·96 | 30 | 1 : 32 |
| 11—12 | 41 | 22·9 | 0·77 | 26 | 1 : 29 |
| 12— 1 p.m. | 36 | 24·5 | 0·56 | 28 | 1 : 20 |
| 1— 2 | 42 | 26·0 | 0·79 | 42 | 1 : 19 |
| 2— 3 | 52 | 19·7 | 1·17 | 55 | 1 : 21 |
| 3— 4 | 51 | 28·5 | 1·39 | 61 | 1 : 23 |
| 4— 5 | 76 | 41·0 | 1·83 | 76 | 1 : 24 |
| 5— 6 | 52 | 24·1 | 1·14 | 24 | 1 : 48 |
| 6— 7 | 75 | 28·0 | 1·35 | 27 | 1 : 49 |
| 7— 8 | 44 | 34·7 | 1·57 | 31 | 1 : 49 |
| 8— 9 | 26 | 20·3 | 0·79 | 14 | 1 : 56 |
| 9—10 | 37 | 28·1 | 1·43 | 27 | 1 : 52 |
| 10—11 | 25 | 17·5 | 0·95 | 14 | 1 : 68 |

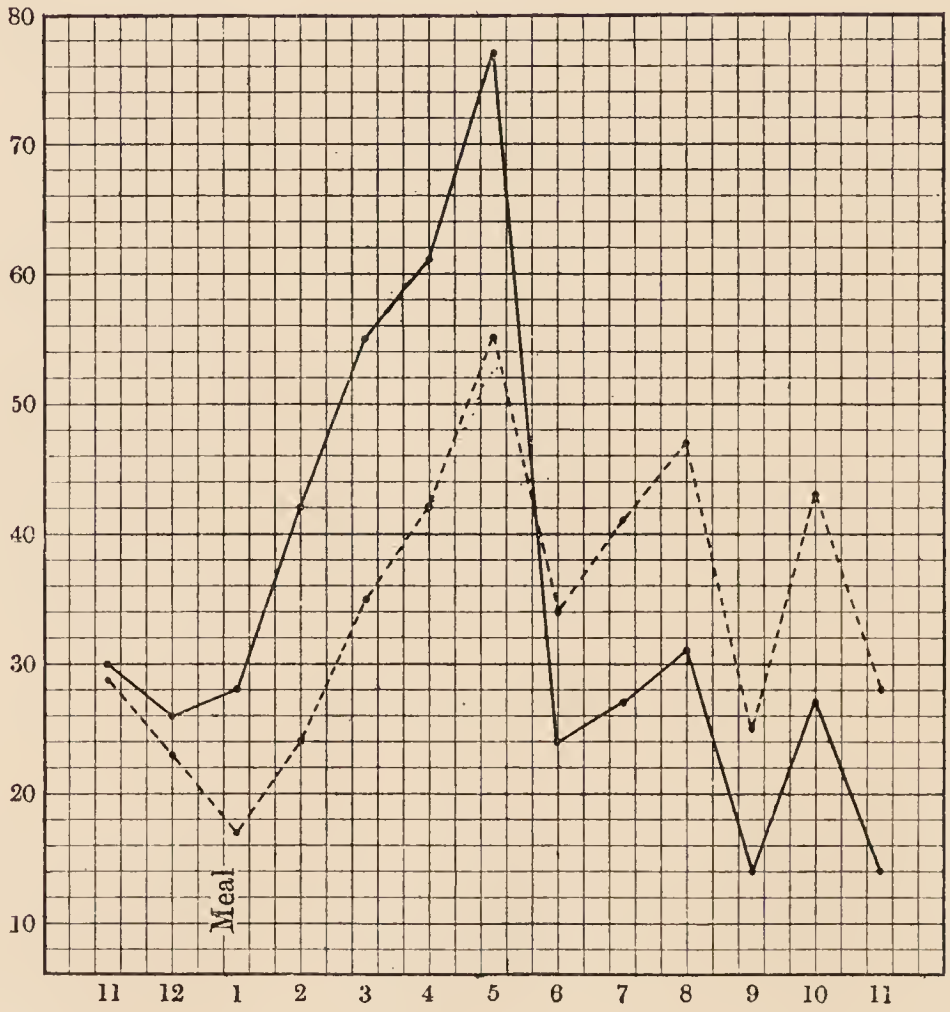


Fig. 5. Urea and Uric Acid Curves. Exp. III.

The next experiment shows much the same results as Experiment I. The urea figures were obtained by the Mörner-Sjöqvist process.

Exp. IV. (F. H. July 23, 1896.) Abstained from 7 p.m. July 22. At 1 p.m. July 23, took 200 grms. lean beef (weighed raw) and 100 grms. bread. Total N of meal by Kjeldahl 7·2 grms.

| Hour | Quantity of urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio uric acid: urea |
|---|---|---|---|---|
| 11—12 a.m. | 46 | 0·81 | 23 | 1 : 35 |
| 12— 1 p.m. | 42 | 0·79 | 23 | 1 : 34 |
| 1— 2 | 27 | 0·61 | 22 | 1 : 28 |
| 2— 3 | 59 | 0·88 | 40 | 1 : 22 |
| 3— 4 | 45 | 0·89 | 43 | 1 : 21 |
| 4— 5 | 100 | 1·40 | 45 | 1 : 31 |
| 5— 6 | 151 | 1·62 | 31 | 1 : 52 |
| 6— 7 | 77 | 1·23 | 27 | 1 : 46 |
| 7— 8 | 56 | 1·21 | 28 | 1 : 43 |
| 8— 9 | 80 | 1·22 | 24 | 1 : 51 |
| 9—10 | 103 | 1·21 | 20 | 1 : 60 |

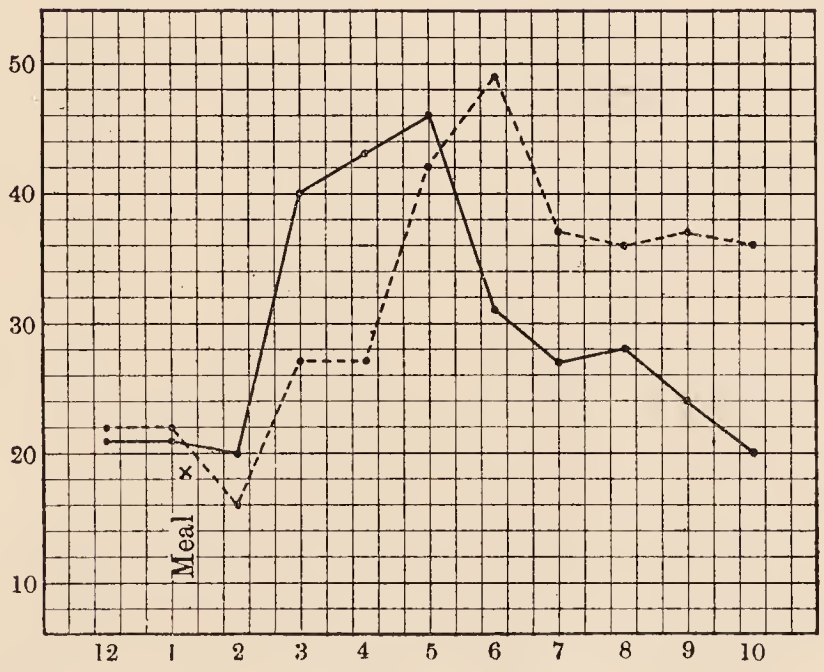


Fig. 6. Urea and Uric Acid Curves. Exp. IV.

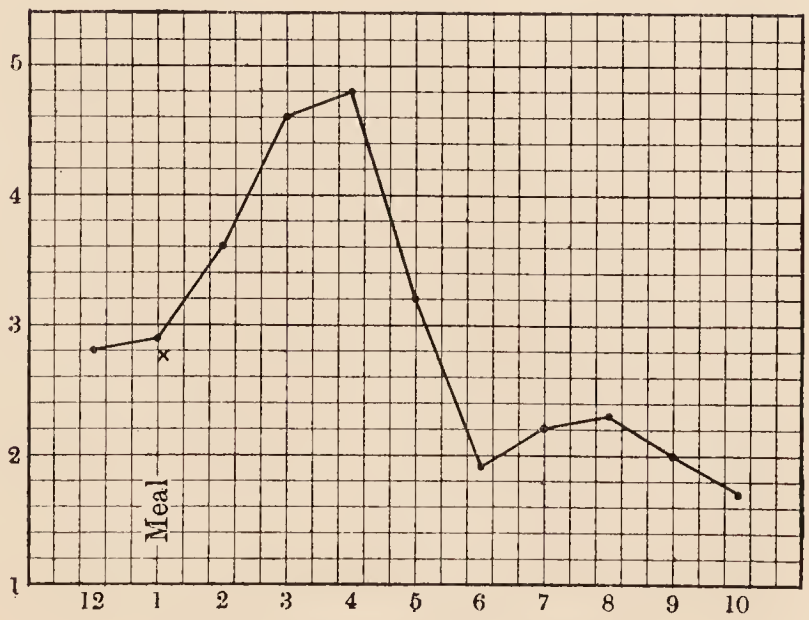


Fig. 7. Ratio Curve. Exp. IV.

In Experiment V. a meal was taken which in proportion to the habits and body weight of the subject was a heavy one. The increase in urea was great and the proportionate amount of uric acid somewhat low throughout, but although the excretion was not followed after the 8th hour the characteristic precedence of the latter constituent is well seen.

Exp. V. (H. N. April 4, 1897.) Abstained from 8 p.m. April 3. Between 1 and 1.30 p.m. April 4, took 280 grms. cooked lean beef, and 150 grms. bread. 100 c.c. of water was drunk during each hour of the experiment.

| Hour | Quantity of urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio uric acid : urea |
|---|---|---|---|---|
| 10.30—11.30 a.m. | 36 | 1·04 | 32 | 1 : 32 |
| 11.30—12.30 p.m. | 34 | 0·94 | 25 | 1 : 38 |
| 12.30—1.30 | 33 | 1·08 | 24 | 1 : 45 |
| 1.30—2.30 | 29 | 1·16 | 33 | 1 : 35 |
| 2.30—3.30 | 45 | 1·61 | 55 | 1 : 29 |
| 3.30—4.30 | 71 | 2·15 | 70 | 1 : 30 |
| 4.30—5.30 | 89 | 2·27 | 63 | 1 : 36 |
| 5.30—6.30 | 70 | 2·42 | 58 | 1 : 42 |
| 6.30—7.30 | 103 | 3·00 | 60 | 1 : 50 |
| 7.30—8.30 | 55 | 1·67 | 32 | 1 : 52 |
| 8.30—9.30 | 50 | 1·78 | 27 | 1 : 66 |

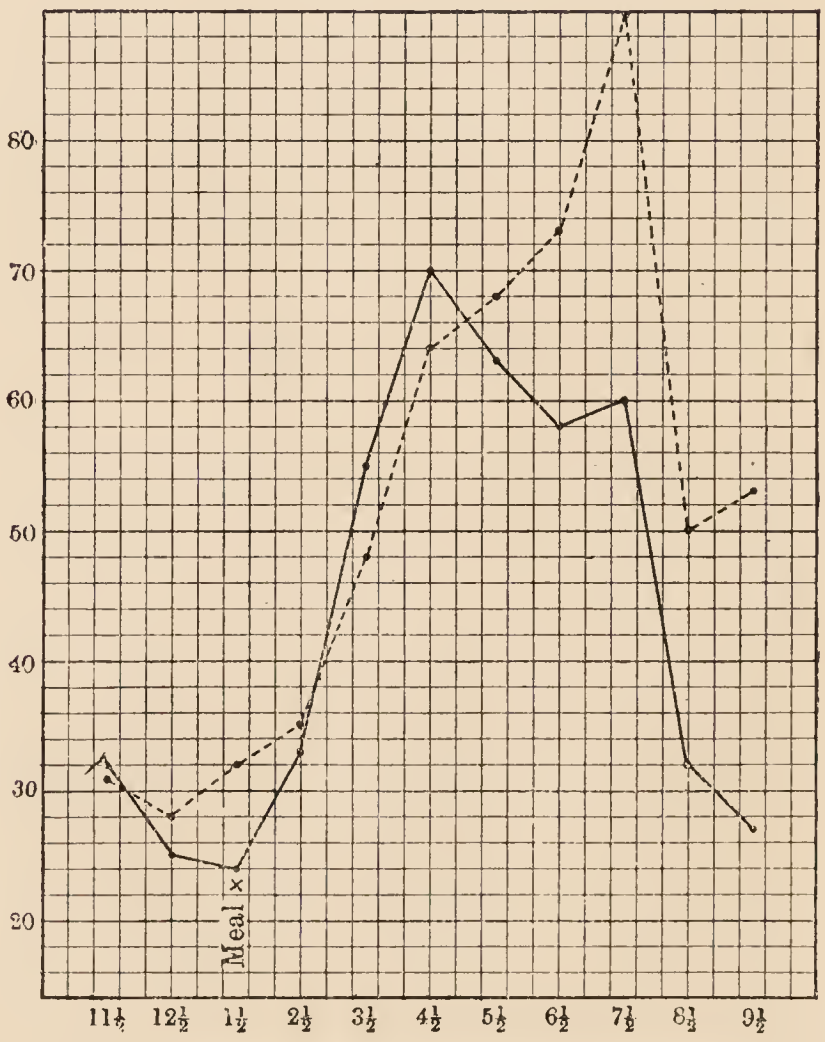


Fig. 8. Urea and Uric Acid Curves. Exp. V.

The following experiment is not typical in that the urea is for some reason very little affected by the meal and the ratio of uric acid to the urea is high throughout. The early rise of the former is however well seen, and towards the close of the experiment it is falling while the urea tends to rise. The ratio curve shows a marked rise and fall.

Exp. VI. (S.) Meal of mixed diet taken at 12.30 p.m. after 14 hours' abstinence.

| Hour | Quantity of urine in c.c. | Acidity (c.c. of N/10 alkali neutralized) | Urea in grms. | Uric acid in milligrms. | Ratio uric acid : urea |
|---|---|---|---|---|---|
| 10—11 a.m. | 40 | 21·6 | 1·15 | 38 | 1 : 30 |
| 11—12 | 39 | 24·2 | 1·15 | 44 | 1 : 26 |
| 12— 1 p.m. | 25 | 16·0 | 0·62 | 22 | 1 : 28 |
| 1— 2 | 35 | 28·7 | 0·99 | 37 | 1 : 26 |
| 2— 3 | 38 | 36·5 | 1·34 | 65 | 1 : 21 |
| 3— 4 | 40 | 35·2 | 1·22 | 68 | 1 : 18 |
| 4— 5 | 19 | 17·1 | 0·55 | 38 | 1 : 14 |
| 5— 6 | 39 | 28·8 | 0·94 | 58 | 1 : 16 |
| 6— 7 | 34 | 31·9 | 1·14 | 49 | 1 : 23 |
| 7— 8 | 30 | 24·0 | 0·86 | 40 | 1 : 22 |
| 8— 9 | 29 | 26·1 | 0·95 | 32 | 1 : 29 |
| 9—10 | 34 | 30·6 | 0·96 | 40 | 1 : 24 |
| 10—11 | 27 | 24·3 | 1·03 | 31 | 1 : 33 |
| 11—12 | 28 | 32·1 | 1·15 | 28 | 1 : 41 |

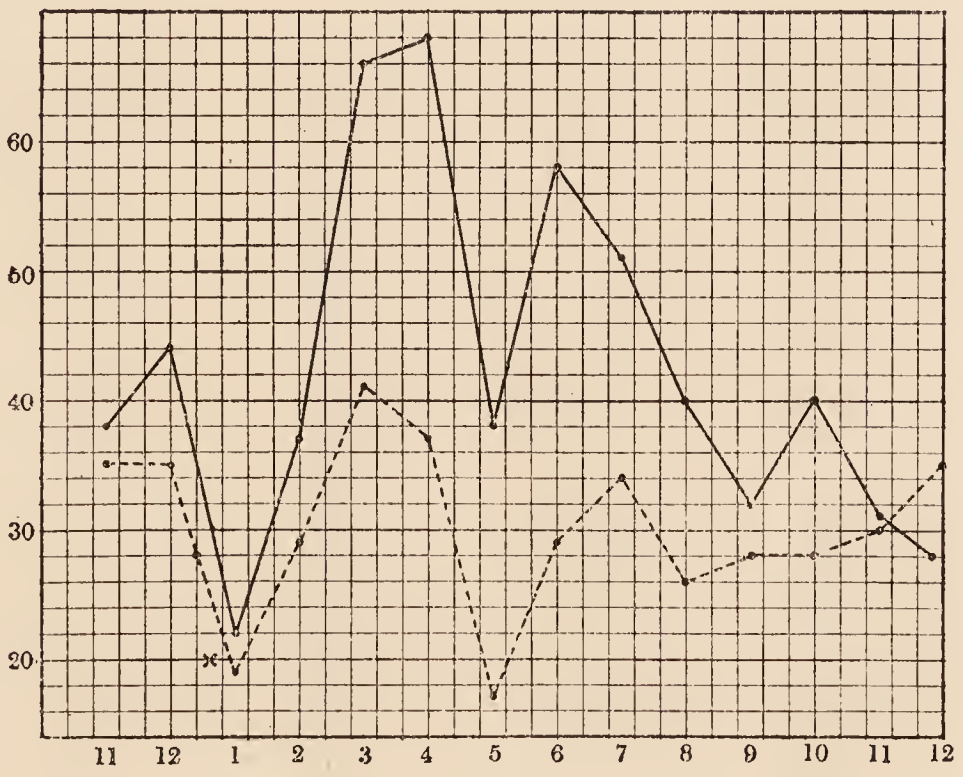


Fig. 9. Urea and Uric Acid Curves. Exp. VI.


Fig. 10. Ratio Curve. Exp. VI.

The two following experiments complete the series. The first shows results closely resembling those of Exp. I., and the second agrees with Exp. III.

Exp. VII. (E. F. G. April 14, 1897.) Fasted for 14 hours before taking test meal of mixed diet at 1 p.m.

| Hour | Quantity of urine in c.c. | Acidity | Urea in grms. | Uric acid in milligrms. | Ratio uric acid: urea |
|---|---|---|---|---|---|
| 10—11 a.m. | 75 | 21·6 | 0·75 | 20 | 1 : 37 |
| 11—12 | 70 | 21·6 | 0·78 | 22 | 1 : 35 |
| 12— 1 p.m. | 45 | 20·0 | 0·90 | 21 | 1 : 43 |
| 1— 2 | 40 | 24·0 | 0·91 | 34 | 1 : 27 |
| 2— 3 | 52 | 36·0 | 0·91 | 39 | 1 : 23 |
| 3— 4 | 70 | 37·0 | 1·01 | 54 | 1 : 19 |
| 4— 5 | 65 | 31·0 | 1·30 | 40 | 1 : 32 |
| 5— 6 | 60 | 29·0 | 1·91 | 44 | 1 : 43 |
| 6— 7 | 64 | 27·0 | 1·65 | 39 | 1 : 43 |
| 7— 8 | 61 | 21·6 | 1·11 | 29 | 1 : 38 |
| 8— 9 | 48 | 26·2 | 1·35 | 25 | 1 : 54 |
| 9—10 | 36 | — | 0·91 | 25 | 1 : 36 |
| 10—11 | 28 | 30·2 | 1·02 | 18 | 1 : 57 |

Exp. VIII. (P. Jan. 19, 1898.) Fasted for 13 hours before taking test meal of beef-steak and bread at 1 p.m.

| Hour | Quantity of urine in c.c. | Acidity | Urea in grms. | Uric acid in milligrms. | Ratio uric acid : urea |
|---|---|---|---|---|---|
| 10—11 a.m. | 48 | 20·0 | 0·98 | 30 | 1 : 27 |
| 11—12 | 45 | 17·2 | 0·77 | 29 | 1 : 27 |
| 12— 1 p.m. | 38 | 17·5 | 0·68 | 27 | 1 : 25 |
| 1— 2 | 46 | 21·0 | 0·91 | 39 | 1 : 23 |
| 2— 3 | 71 | 28·3 | 0·98 | 51 | 1 : 19 |
| 3— 4 | 46 | 28·1 | 1·31 | 62 | 1 : 21 |
| 4— 5 | 47 | 31·0 | 1·76 | 64 | 1 : 27 |
| 5— 6 | 56 | 27·4 | 1·08 | 27 | 1 : 40 |
| 6— 7 | 75 | 20·8 | 1·21 | 27 | 1 : 45 |
| 7— 8 | 55 | 21·1 | 1·59 | 32 | 1 : 50 |
| 8— 9 | 51 | 18·0 | 1·16 | 22 | 1 : 53 |
| 9—10 | 38 | 22·1 | 1·20 | 24 | 1 : 50 |
| 10—11 | 21 | 18·0 | 0·91 | 21 | 1 : 43 |

## II. Does the rise of uric acid which follows a meal represent the accelerated excretion of preformed material?

It has been assumed that the body normally contains a store of retained urates upon which digestion may exercise some indirect influence—as, for instance, in its induction of an "alkaline tide[1]." It may be held therefore that the increase of uric acid which appears after a meal represents preformed material brought to excretion as a secondary effect of the food ingestion. Although this view involves pure assumptions it is necessary to refer to it in this place, as, were it tenable, it might easily explain the phenomenon under discussion, and would of course leave the experiments of the previous section without any bearing upon the mode of production of uric acid. The "alkaline tide" of Bence Jones and Roberts occurs for instance at a quite early period after a meal, and upon the above view might be responsible for the early outflow of uric acid.

But very few determinations of the urinary variants after a meal suffice to show that an alkaline tide is by no means an universal accompaniment of digestion; and under the conditions of our present experiments it appears to be even an exceptional phenomenon. In some cases the urine it is true became alkaline or had its acidity diminished during the period of maximum uric acid excretion. Any

[1] Haig, Uric acid and Disease.

such phenomena may of course happen to the parallel, but that no causal relation obtains between them is abundantly shown by the figures of Experiments III., VI., VII., and VIII. in the previous section. In these it will be seen that the typical early excretion of uric acid occurs, not only in the complete absence of any urinary alkaline tide, but with a concurrent increase of acidity.

Another factor which ought perhaps to be considered—if the possibility of any important retention of urates in the normal organism be entertained at all—is the conceivable influence upon their excretion which might be exerted by temporary variations in the neutral salts of the blood resulting from food ingestion.

The very conclusive experiments of Sir Wm Roberts which show that the presence of sodium salts (whether the alkaline carbonate or neutral salts of this metal) actually diminish the solubility of sodium biurate in any medium are evidence against the ingestion of salts of soda being likely to accelerate the excretion of retained urates. These experiments are in accord with a physico-chemical principle since enunciated and illustrated experimentally by Nernst, which can be shown to be of very general application. Nernst's generalization is to the effect that any two salts susceptible of electrolytic dissociation which contain an electric ion in common mutually diminish each other's solubility. We might expect *a priori* therefore that any sodium compound capable of electrolytic dissociation would, if present in a menstruum, diminish the solubility of sodium urate in that menstruum. But the converse of this proposition has been found to hold true—at least in certain cases—and salts possessing no electric ion in common may mutually increase each other's solubility in a fluid. The possibility exists therefore that the ingestion of a mixed dietary may produce such a temporary increase in the proportion of salts other than those of sodium (especially of potassium salts) as to increase the solubility of any retained sodium urates and so to accelerate its excretion.

V. Bunge long ago showed the important influence that the ingestion of potassium salts can exercise in removing sodium from the blood; and, even if it be true—as the experiments of Roberts and Luff seem to show—that an increase of potassium salts in a menstruum such as the plasma would not directly assist the solubility of sodium urate, the temporary reduction of sodium salts produced by taking potassium must by itself tend to do so. Now the salts of a mixed dietary contain a larger proportion of potassium than of sodium, and it is not inconceivable that some effect upon retained urates might arise from the

salts of a meal acting on the lines just discussed, in temporarily disturbing the balance of sodium salts in the plasma.

Whatever weight such *a priori* considerations may be thought to carry (and it is not unlikely that they may rise to importance in conditions of lithaemia) we may say at once that from our own experiments we believe that no such influence of salts is to be observed in conditions of normal health, probably because as a matter of fact there is so little retained sodium urate present upon which any such influence could be exerted.

The administration of various salts during abstinence, either alone or added to test meals of eggs or milk, has failed with us to produce any rise of uric acid at all comparable with that seen after meals of ordinary mixed dietary.

That diuresis is in no way responsible for the early excretion of uric acid after a meal will be evident on inspecting the protocols of the experiments in the first section; the phenomenon occurs regularly in spite of great variations in the quantity of urine excreted hour by hour, and is seen in cases where no diuresis follows the meal.

Some evidence against the early excretion being due to any secondary influence whatever of the meal upon preexisting urates is found in the fact that if a second meal be taken, equivalent to the first, at a time when the uric acid tide of the latter is falling (6th or 7th hour of digestion) an equivalent second rise occurs. If the first rise had been due to a clearing out of the more available urates accumulated during abstinence it is reasonable to suppose that the second would be proportionately small.

## III. The relation of the post-prandial rise to digestion-leucocytosis.

We referred in the introductory paragraphs of this paper to certain difficulties in the way of accepting Horbaczewski's hypothesis of the exclusive origin of uric acid from the leucocytes of the blood. As regards post-prandial phenomena it is clear of course that the leucocytosis and the uric acid increase must often occur together; but an examination of the experimental evidence available will show that exceptions to this parallelism are too frequent for a direct causal relation to be likely.

Even in Horbaczewski's original work certain anomalies were

observed by him, though he held them capable of explanation. Experimental leucocytosis comparable in degree with digestive leucocytosis was found to have very much less effect upon uric acid. Horbaczewski says: "One cannot conceal from oneself that at times a certain disproportion (between leucocytosis and uric acid excretion) occurs. After taking pilocarpin or nuclein a very marked leucocytosis takes place, as after the ingestion of large amounts of flesh food. The uric acid increase associated with the former is however not very important[1], while after flesh food it is more lasting and much better marked." To explain this, Horbaczewski suggests that qualitative differences may exist in the nature of the leucocytes in the two cases, but he adduces no experimental support of this. Again, in studying the effect of other drugs he found that antipyrine and antifebrine produced a temporary leucocytosis comparable with that due to a meal, but associated with a decrease instead of an increase in the urinary uric acid. The explanation offered in this case is that while pilocarpin produces the leucocytosis by increasing the cells, the other drugs bring about the effect by a preservation of leucocytes; by a prevention of the normal destruction. This might perhaps be tested by a simultaneous study of the phosphorus excretion, but we believe no such evidence is to hand. To accept Horbaczewski's theory we have thus to admit certain conceptions which are certainly not upon an established experimental basis.

We have already referred to the evidence from such a dietary as egg-white, which, while quite capable of producing a digestive leucocytosis, has little or no effect upon uric acid excretion. We shall now give two experiments of our own made upon diet as free as possible from nitrogen. The results of these fully confirm Camerer's[2] observations in showing that meals of this character depress rather than raise the uric acid output, whereas they show a decided effect in increasing the circulating leucocytes.

Even if the increase of circulating leucocytes during digestion could be shown to be due always to increased formation of these cells and not, as it is at least possible, to a chemiotatic and merely distributive

[1] Other observers have found that small quantities of pure nuclein may induce a leucocytosis without observable effect on the uric acid. Paul Meyer, *Deutsch. med. Wochenschr.* No. 12, S. 186, 1896; Milroy and Malcolm found with nucleic acid an increase so small as to be negligible, falling as it did within the limits of variations seen in the control period when normal fixed diet was taken, *loc. cit.* p. 228.

[2] *Ztschr. f. Biol.* XXXIII. S. 136. 1896.

influence, such observations as the following show that a digestive leucocytosis, equal in degree to what is ordinarily described, may occur without any associated rise in uric acid, and seem to us to make it unlikely that any essential relation obtains between them.

Exp. IX. (F. H.) After 14 hours' abstinence a thick water gruel made from 6 ozs. of arrowroot with butter and sugar was taken at 1 p.m. The leucocytes were counted at the end of each two-hourly period.

| Hour | Urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio | Leucocytes |
|---|---|---|---|---|---|
| 11 a.m.—1 p.m. | 102 | 1·56 | 41 | 1 : 38 | 7000 |
| 1—3 | 304 | 1·48 | 38 | 1 : 36 | 6800 |
| 3—5 | 150 | 1·12 | 30 | 1 : 37 | 10500 |
| 5—7 | 168 | 1·38 | 28 | 1 : 49 | 8500 |
| 7—9 | 156 | 1·14 | 27 | 1 : 42 | 8000 |

Exp. X. (F. H.) After 14 hours' abstinence took at 1 p.m. 6 ozs. of arrowroot in the form of a gruel, with 4 ozs. of filtered butter fat and 4 ozs. of cane sugar.

| Hour | Urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio | Leucocytes |
|---|---|---|---|---|---|
| 11 a.m.—1 p.m. | 86 | 1·49 | 42 | 1 : 35 | 8500 |
| 1—3 | 410 | 1·51 | 40 | 1 : 38 | 9500 |
| 3—5 | 200 | 1·09 | 36 | 1 : 30 | 10800 |
| 5—7 | 160 | 1·25 | 28 | 1 : 45 | 8500 |
| 7—9 | 172 | 1·40 | 20 | 1 : 70 | 8500 |

We feel, doubtless in common with other observers, that the present methods of counting leucocytes are very unsatisfactory when only small physiological variations are to be studied; but as such measurements form the basis of the digestive leucocytosis theory of the origin of uric acid it is fair that the above should be considered. The numbers were obtained in each case as the mean of several fairly accordant counts, the blood being obtained simultaneously from the finger and from the lobe of the ear. We do not know whether such meals as the above are in all persons capable of producing a digestive leucocytosis[1], but the fact that in an individual, who after meals of ordinary diet showed a well-marked rise of uric acid (Exps. I., IV. &c.), there was, with non-nitrogenous diet, no rise at all, in spite of a decided digestive leucocytosis, seems strong evidence in favour of referring the excretion directly to the diet and not to any intermediary leucocytosis.

[1] Pohl (*loc. cit.*) found no leucocytosis after the ingestion of carbohydrates and fats.

## IV. Is the post-prandial increase due solely to ingested nucleins?

The evidence so far discussed seems to us to leave little doubt that the uric acid increase which follows a meal represents in the main neither the excretion of preformed material nor the result of a special breakdown of leucocytes. It rather takes origin direct from the ingesta; in at least as strict a sense as the urea can be said to do. We have now to discuss the view that of the ingesta the nucleins are alone responsible for its increase.

This view is based almost entirely upon observations which have shown that while a dietary of eggs or milk scarcely affects the uric acid output, an exceptionally large increase follows the ingestion of thymus gland. It has been generally assumed that the sole reason for this marked difference is to be found in the absence of true nucleins from the former dietary and their exceptional abundance in the latter; ordinary muscle-diet being intermediate, both in its proportion of nucleins and in its effect upon uric acid.

We shall now give the results of experiments which show the effect upon excretion of thymus gland on the one hand and of eggs and milk on the other when taken as isolated test meals. The effect of these special dietaries upon the excretion of uric acid has been fully studied, but not from the standpoint of the experiments detailed in the first section.

Exp. XI. (F. H.) After 18 hours' abstinence a lightly cooked calves' thymus gland weighing when raw 250 grms. was taken at 4 p.m.

| Hour | Urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio |
|---|---|---|---|---|
| 2—3 p.m. | 35 | 0·81 | 18 | 1 : 45 |
| 3—4 | 40 | 0·75 | 17 | 1 : 44 |
| 4—5 | 75 | 0·90 | 19 | 1 : 47 |
| 5—6 | 68 | 1·28 | 29 | 1 : 44 |
| 6—7 | 52 | 1·32 | 54 | 1 : 24 |
| 7—8 | 40 | 1·03 | 60 | 1 : 17 |
| 8—9 | 34 | 1·47 | 37 | 1 : 39 |

It will be seen that in this experiment there is a rise in the uric acid as rapid as after the meals of ordinary diet, a large increase occurring in the 3rd and 4th hours of digestion.

The results of the two experiments which follow are in accord with the observations of others which have shown that egg-proteid and milk have but little effect on the excretion of uric acid.

Exp. XII. (E. L.) After 18 hours' abstinence, took at 1 p.m. 410 grms. egg-white and one pint of milk.

| Hour | Urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio |
|---|---|---|---|---|
| 11—12 a.m. | 40 | 0·78 | 22 | 1 : 36 |
| 12—1 p.m. | 41 | 0·76 | 22 | 1 : 34 |
| 1—2 | 115 | 1·61 | 32 | 1 : 50 |
| 2—3 | 235 | 1·74 | 38 | 1 : 46 |
| 3—4 | 73 | 1·46 | 25 | 1 : 56 |
| 4—5 | 90 | 1·74 | 25 | 1 : 70 |
| 5—6 | 92 | 1·41 | 21 | 1 : 67 |

Though there is some increase in uric acid in the above experiment at the 1st and 2nd hour, it is very small (in spite of much increase in the urinary water) compared with that after meat diet of approximately equal nitrogenous value. The increase of urea is rapid and the ratio (uric acid : urea) is lowered instead of raised.

Exp. XIII. (F. H.) After 14 hours' abstinence, took 480 grms. egg-white at 2 p.m.

| Hour | Uric acid in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio |
|---|---|---|---|---|
| 12—1 p.m. | 70 | 1·14 | 27 | 1 : 42 |
| 1—2 | 72 | 1·11 | 26 | 1 : 43 |
| 2—3 | 232 | 1·40 | 36 | 1 : 39 |
| 3—4 | 71 | 1·11 | 32 | 1 : 35 |
| 4—5 | 40 | 0·97 | 24 | 1 : 40 |
| 5—6 | 272 | 1·50 | 33 | 1 : 45 |
| 6—7 | 34 | 0·85 | 19 | 1 : 45 |
| 7—8 | 67 | 1·19 | 8 | 1 : 149 |
| 8—10 | 43 | 0·61 | 15 | 1 : 40 |
| (2 hours) | | (mean per hr.) | (mean per hr.) | |
| 10—11 | 16 | 0·48 | 8 | 1 : 60 |

In Experiment XIII. as in the previous one, the rise in uric acid is very small, though a large diuresis occurred after taking the egg-white. During the later hours of this experiment the output of both excretives became very low and at the same time some discomfort was experienced.

A study of excretion after isolated meals shows therefore, no less than the whole 24 hours' metabolism is dealt with, that egg-white has at the most a very slight influence upon the output of uric acid, and differs markedly in this respect from thymus-diet or ordinary muscle-diet.

But clearly other constituents are found in such an organ as the thymus (and in muscle) which are lacking from egg-proteid and from

milk; and it is important in the present state of knowledge not to neglect the possibility that certain of the more soluble constituents of the gland or muscle may act as precursors of uric acid. The proportionately rapid elimination after a meal, so difficult to reconcile with an origin from the indigestible nucleins would be explained if any soluble and diffusible constituent of the diet is capable of acting as a precursor, either directly, or as a factor in a synthetic process.

It should be carefully observed that the view of the importance of nucleins is almost entirely based upon the effects of thymus feeding; when pure nucleins have been administered the results have been by no means convincing or concordant.

As stated above Horbaczewski himself found that pure nuclein, though in his hands (in one experiment at any rate) it produced a definite rise, did not affect the uric acid output in nearly so great a proportion as did a rich meal of flesh-diet. Now the proportion of nucleo-proteids in muscle is very small. Pekelharing[1] obtained 2 grammes of nucleo-proteid from 543 grammes of dog's muscle, and from 300 grammes of rabbit's muscle he prepared 0·1552 grammes of nuclein. This observer states that he obtained similar values for ox muscle, and we may fairly conclude from his results that raw beef, even allowing for the blood present, cannot contain more than at most 0·1 per cent. of nuclein. Now it has been found by Paul Meyer (*loc. cit.*) that pure nuclein taken by the mouth, in the form of capsules containing 2 grammes each, produced no effect whatever upon uric acid excretion. This result was attributed to the smallness of the quantity taken; but, assuming Pekelharing's figures to be approximately correct, something like two kilos of flesh would have to be taken to represent 2 grammes of nuclein. Even if the amount in raw beef were considerably in excess of this, it is clear that such quantities (circa 200 grammes) as were taken in our isolated meal experiments, producing a marked increase in uric acid, would contain much smaller quantities than 2 grammes of nuclein.

Other observers have found no rise at all after the ingestion of large quantities of nuclein and nucleic acid. Thus Stadthagen[2] gave yeast-nuclein to dogs without effect upon their uric acid, and Gumlich[3] also employing dogs administered large quantities of nucleic acid (22 grammes) with absolutely negative results. The dog is not a

[1] *Zeit. f. physiol. Chem.* XXII. S. 247.
[2] *Virchow's Archiv*, CIX. S. 390.
[3] *Zeitsch. f. physiol. Chem.* XVIII. S. 508.

wholly satisfactory animal on which to test this question owing to its very low normal output of uric acid. The excretion is however by no means absent from its urine as sometimes stated[1].

It occurred to us that by submitting thymus gland to artificial gastric digestion, and by taking as test meals the filtered extracts, we might be able to eliminate the nucleins, while obtaining in solution other substances possibly capable of acting as uric acid precursors.

As a matter of fact such pepsin-hydrochloric acid extracts of the gland, filtered till perfectly clear, and boiled down to small bulk (after neutralizing with sodium carbonate), produce an abundant and rapid increase of uric acid. Egg-white digested in like manner as a control and similarly treated, produced only the usual minimal rise which follows its ingestion under ordinary circumstances.

Exp. XIV. (W. H.) Four calves' thymus glands finely cut up were digested for 10 hours at 35° with 0·4 per cent. HCl and a very active pepsin preparation. The extract was squeezed through calico, and then filtered through paper till perfectly clear. Half of the whole preparation was neutralized with sodium carbonate, concentrated, and taken as a test meal after 14 hours' abstinence.

| Excretion of: | Urine in c.c. | Urea in grms. | Uric acid in milligrms. | Ratio |
|---|---|---|---|---|
| 2 hours before taking extract | 80 | 2·03 | 43 | 1 : 47 |
| 1st & 2nd hrs. after | 160 | 2·51 | 205 | 1 : 12 |
| 3rd & 4th hrs. after | 140 | 3·07 | 169 | 1 : 18 |

Exp. XV. (F. H.) Six glands treated as in XIV. and one-third of the extract taken after 14 hours' abstinence.

| Excretion of: | Urine in c.c.[2] | Urea | Uric acid | Ratio |
|---|---|---|---|---|
| 2 hours before | 132 | 1·34 | 34 | 1 : 40 |
| 1st & 2nd after | 280 | 2·66 | 107 | 1 : 25 |
| 3rd & 4th „ | 445 | 2·89 | 126 | 1 : 23 |
| 5th & 6th „ | 104 | 1·76 | 32 | 1 : 55 |

[1] Cf. Huppert, *Neubauer and Vogel. Analyse des Harns*, xth auflage, p. 328.

[2] That the diuresis seen in these experiments could not by itself account for the rise in uric acid is negatived by the results in earlier parts of the paper. Compare especially Expts. XII. and XIII.

Exp. XVI. (S. N. P.) The pepsin-HCl extract of one gland after 6 hours' digestion was taken after 7 hours' abstinence.

| Excretion of: | Urine in c.c. | Urea | Uric acid | Ratio |
|---|---|---|---|---|
| 2 hours before | 200 | 1·98 | 42 | 1 : 47 |
| 1st & 2nd after | 260 | 2·40 | 98 | 1 : 24 |
| 3rd & 4th ,, | 290 | 2·50 | 96 | 1 : 26 |
| 5th & 6th ,, | 104 | 1·86 | 50 | 1 : 37 |

An obvious objection to these otherwise very striking results lies in the possibility that some proportion of the nucleo-proteids, or of their decomposition products, may go into solution in spite of the action of the pepsin-hydrochloric acid.

Bokay[1] found that no nuclein was taken up during artificial gastric digestion, and Popoff[2] confirmed his results in a careful research upon the digestion of thymus tissue. Milroy[3] on the other hand found, as the result of an experiment on nuclein derived from the gland, that appreciable quantities of phosphorus were dissolved in organic combination by pepsin-hydrochloric acid, though he did not find nuclein or nucleic acid in the extract.

It is somewhat difficult to decide *a priori* what period of digestion would be likely to prove most efficacious in eliminating nuclein products. Prolonged extraction with the acid is perhaps more likely to dissolve some nucleo-proteid, if the percentage acidity be sufficiently high; on the other hand a longer exposure to the ferment is more likely to split off efficiently the insoluble nuclein.

We believe, however, that the evidence directly obtained by testing the pepsin-hydrochloric acid extracts actually employed for the above experiments was sufficient to show that they could have contained no more than minute quantities of nucleo-proteid, of nuclein, or of nucleic acid.

In each experiment much larger quantities of material were digested than were required for the test meal, and a part of each extract was tested as follows: (*a*) In one portion of the clear solution the acidity was reduced to a minimum, and it was then allowed to stand; afterwards the free hydrochloric acid was replaced by free acetic (by the addition of a little sodium acetate). In no case did any phosphorus-containing precipitate fall. (*b*) A portion was allowed to stand over for some hours at 38° C. with further addition of active pepsin solution. In all cases the

[1] *Zeitsch. f. physiol. Chem.* I. S. 157.
[2] *Ibid.* XVIII. S. 533.
[3] *Ibid.* XXII. S. 317.

extract remained perfectly clear; no trace of insoluble nuclein appearing. When to the same portion a little nucleo-proteid in solution was subsequently added a precipitate of nuclein rapidly formed. (*c*) Presumptive evidence against the presence of nuclein or nucleic acid in appreciable quantity was obtained from the fact that the filtered peptic extracts immediately precipitated these substances when solutions of either were added to them in small quantities. Thus a few drops of a solution of thymus-nuclein produced an immediate precipitate when added to the clearer digested extract, and a solution of nucleic acid, prepared from the gland by Kossel's method, was wholly precipitated when mixed with a clear extract and allowed to stand. (*d*) A mixture made from portions of each of the extracts employed in the above experiments was treated as in Kossel's method for preparing nucleic acid from aqueous extracts of the gland. No phosphorus-containing product was obtained. (*e*) Finally, to a portion of each preparation tannic acid was added in excess, and the phosphorus determined in the resulting precipitate—a method employed by Popoff. In an amount of material equivalent to that taken in Experiment XV, and from the same digestion extract, 12 milligrammes of phosphorus were present in the precipitate produced by tannic acid. Now it is difficult to wash this precipitate thoroughly free from inorganic phosphates, and a certain proportion of the organic phosphorus was probably not derived from nucleo-proteids. But, on the basis of Kossel's determination of phosphorus in the nucleic acid of thymus gland, the whole of the 12 milligrammes would correspond to about 16 milligrammes of allox-uric nitrogen only, and on the very unlikely assumption that the whole of this could appear in the urine as uric acid, it would account for but 48 milligrammes of the latter—a quantity much smaller than the increase actually produced in Experiment XV. In other preparations even less phosphorus was found.

In conclusion we give the results of an experiment in which nuclein and gastric extract from the thymus gland were respectively added to the fixed dietary of a control period. The series is but short and relates to one individual only; but the figures support the observations of those who have found no increase of uric acid after taking pure nuclein.

The nuclein taken was prepared by thorough gastric digestion of the nucleo-proteid extracted from fresh glands.

Exp. XVII. (F. H.)

| Date | Urine in c.c. | Urea | Uric acid | Ratio | $P_2O_5$ | Diet |
|---|---|---|---|---|---|---|
| July 25 | 1115 | 24·2 | ·559 | 1 : 43 | — | Fixed diet |
| „ 26 | 1210 | 23·6 | ·540 | 1 : 44 | 2·37 | Fixed diet |
| „ 27 | 1010 | 24·9 | ·810 | 1 : 31 | 2·21 | Fixed diet and pepsin-HCl extr. of 2 thymus glands |
| „ 29 | 980 | 23·2 | ·620 | 1 : 37 | — | Fixed diet |
| „ 30 | 1015 | 24·2 | ·552 | 1 : 44 | 2·83 | Fixed diet and 10 grms. nuclein from thymus |
| Aug. 2 | 1100 | 25· | ·571 | 1 : 44 | 3·01 | Fixed diet and 10 grms. nuclein from thymus |
| „ 3 | 1212 | 26·5 | ·592 | 1 : 43 | 2·29 | Fixed diet |

| | Uric acid | Urea | Ratio | $P_2O_5$ |
|---|---|---|---|---|
| Average of fixed diet excretion | ·578 | 24·1 | 1 : 41·7 | 2·33 |
| „ „ and 10 grms. nuclein | ·561 | 24·6 | 1 : 43·8 | 2·92 |
| Fixed diet and gastric extract | ·810 | 24·9 | 1 : 30·8 | 2·21 |

There can be no doubt, after consideration of the available experimental evidence, that the degree in which uric acid production is influenced by diet, depends very directly upon the nature of the ingesta. The marked difference found by all observers in the effect of eggs and milk on the one hand, and of muscle, or especially of thymus gland, on the other, together with the absence of any increase after non-nitrogenous diet is sufficient proof of this. It is we think equally clear that the differences do not depend upon the relative power of different dietaries to produce varying degrees of digestion-leucocytosis. At the same time we believe that the experimental evidence we have brought forward should lead to a revision of the prevalent view that the difference is due entirely to the varying proportion of nucleo-proteids which these dietaries contain.

Such observations as are described in this paper do not, it is true, negative the possibility that of the total production of uric acid in the body some part may be due to the direct breakdown of nucleins—the bearing of our experiments is upon that part of the process alone which results in a rapid increase after food ingestion. In any study of alloxuric excretion the great importance of the central fact that the nuclein-conjugates contain the alloxuric group, or a precursor of this, within their molecule, cannot of course be overlooked; but we think the present tendency to ascribe all uric acid production in the mammal

to the breakdown of these special substances is based on too narrow a view. It involves at least a belief in a very abrupt breach of continuity in the phenomena of comparative physiology, for there is no reason to suppose that the metabolic products which leave the tissues of birds and mammals differ in any fundamental way. The difference in the actual excretive is probably due to quite final hepatic (or renal) influences. It seems unreasonable to assume—unless upon very conclusive experimental evidence—that of the uric acid excreted by the mammal no part represents the product of similar influences, but is to be wholly ascribed to the breakdown of nucleins.

It is the sole purpose of the present paper to point out that the experimental evidence by no means makes for this conclusion, at least as regards one portion of the daily excretion. The probable origin of the uric acid which is so rapidly excreted after a meal we hope to discuss in a future paper.

## Summary.

Experiments on seven individuals confirm the statement of Mareš, that during the period of increased nitrogen excretion which follows a meal, the increase of uric acid has a briefer duration than the increase of urea, and occurs characteristically in the earlier hours of the hyperexcretory period; this fact is difficult to reconcile with the view that the uric acid takes origin from the nucleins of the diet, upon which the earlier stages of digestion have only a minimal influence.

The chief evidence for the view that nucleins play a predominant rôle as uric acid precursors is based upon the results of thymus-feeding. Experiments detailed in the paper show that extracts may be prepared from this gland which contain at most traces of nucleins or nucleic acid, but which when ingested produces the characteristically large excretion of uric acid.

It is therefore suggested that of the total quantity of uric acid normally excreted, that portion which bears a more immediate relation to food does not arise from nucleins but from some more soluble constituent of the diet acting either as a direct precursor or as a factor in a synthetic process.